

FIG. 1

FIG. 2

Sheet 3 of 27

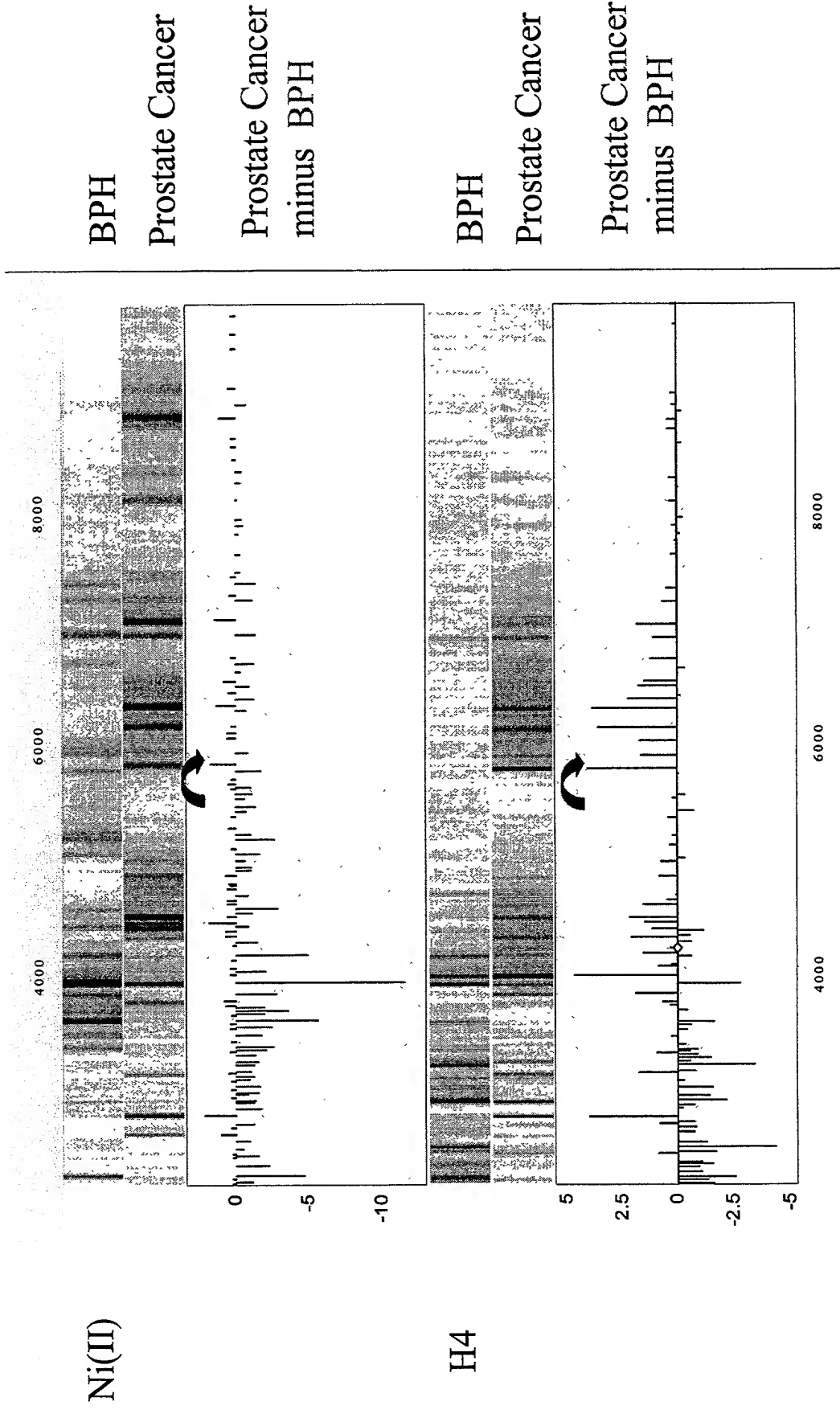


FIG. 3

Sheet 4 of 27

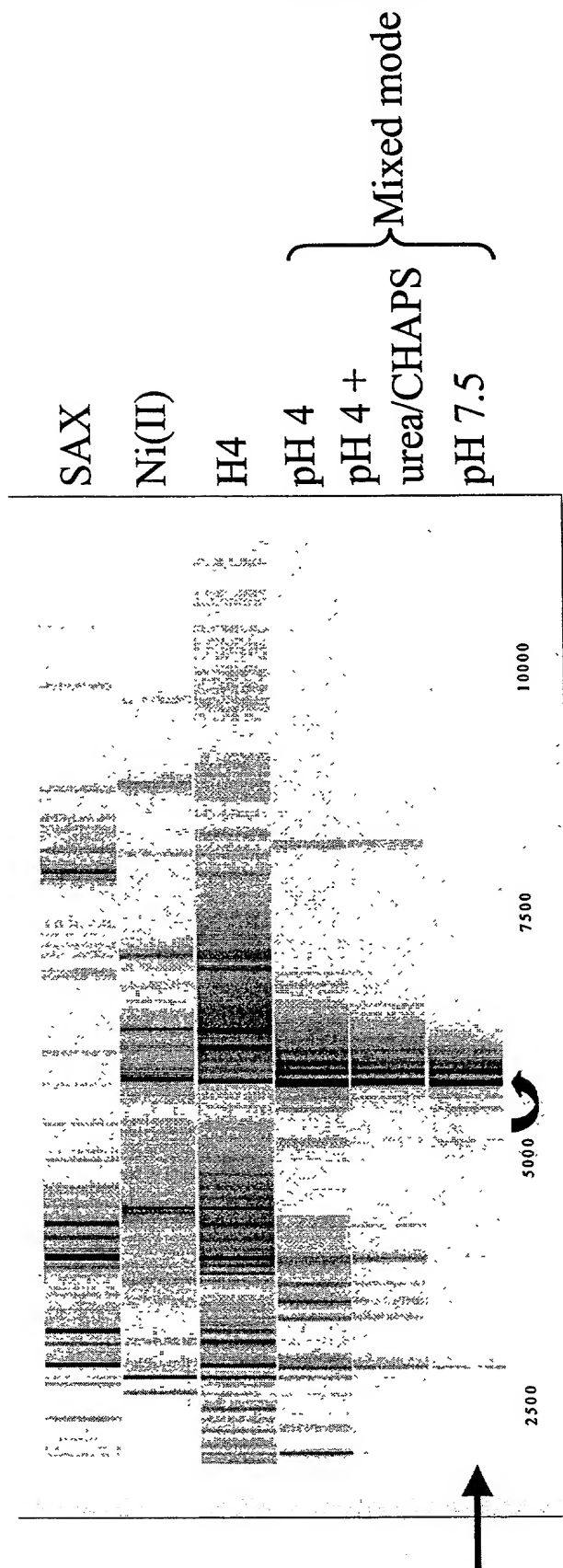


FIG. 4

Sheet 5 of 27

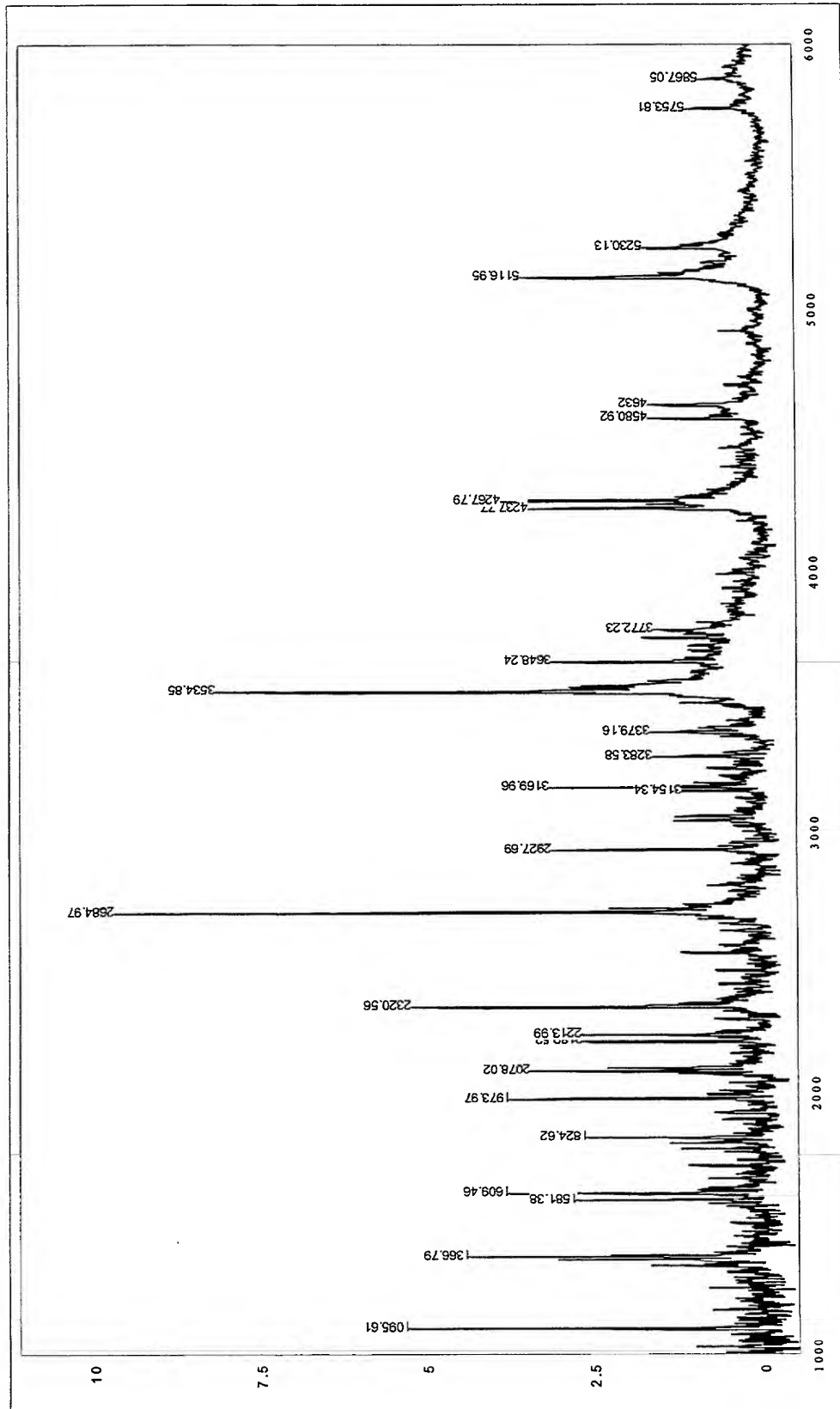


FIG. 5

10066359.013400

Sheet 6 of 27

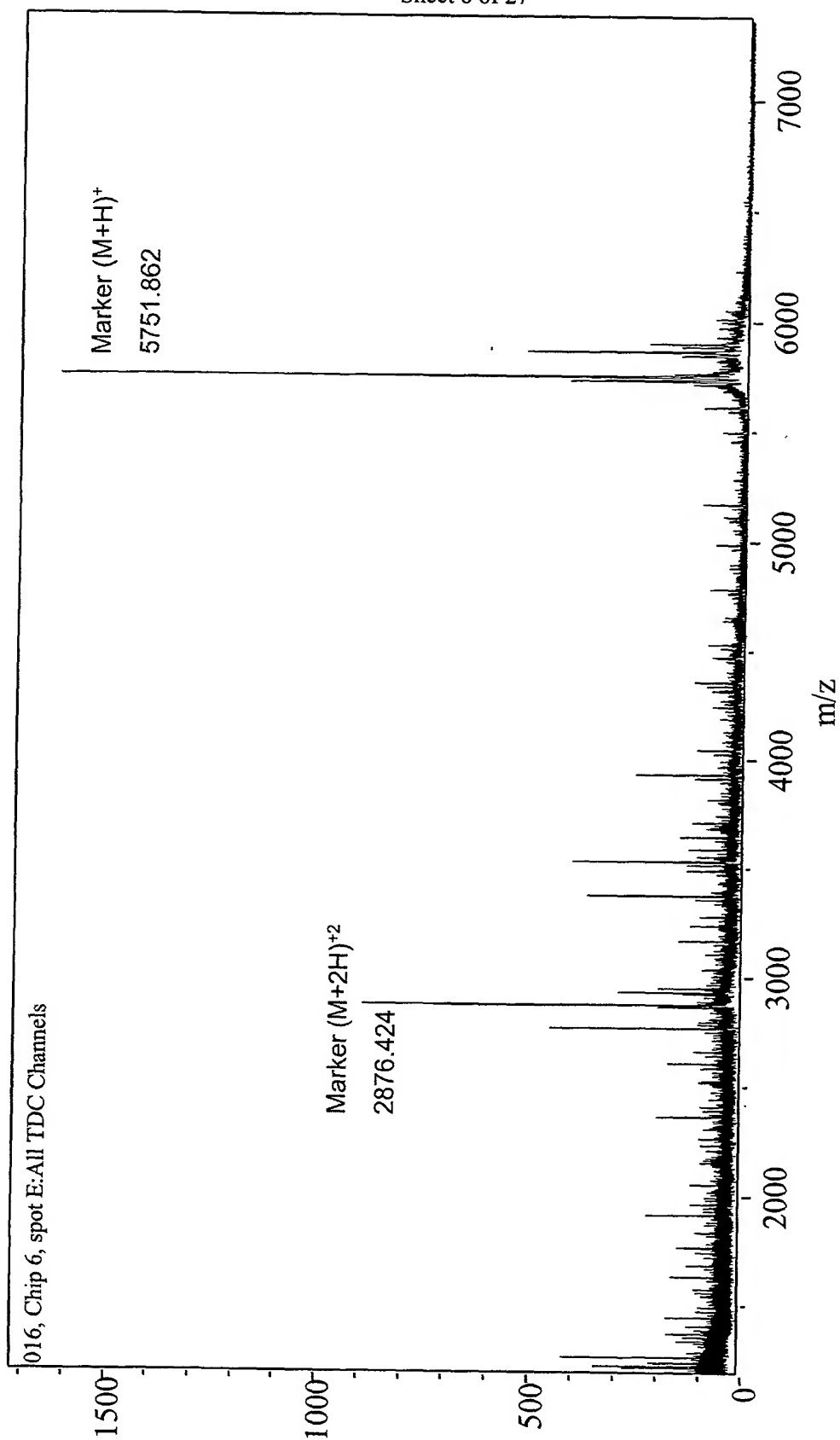


FIG. 6

20161010 "66699001"

Sheet 7 of 27

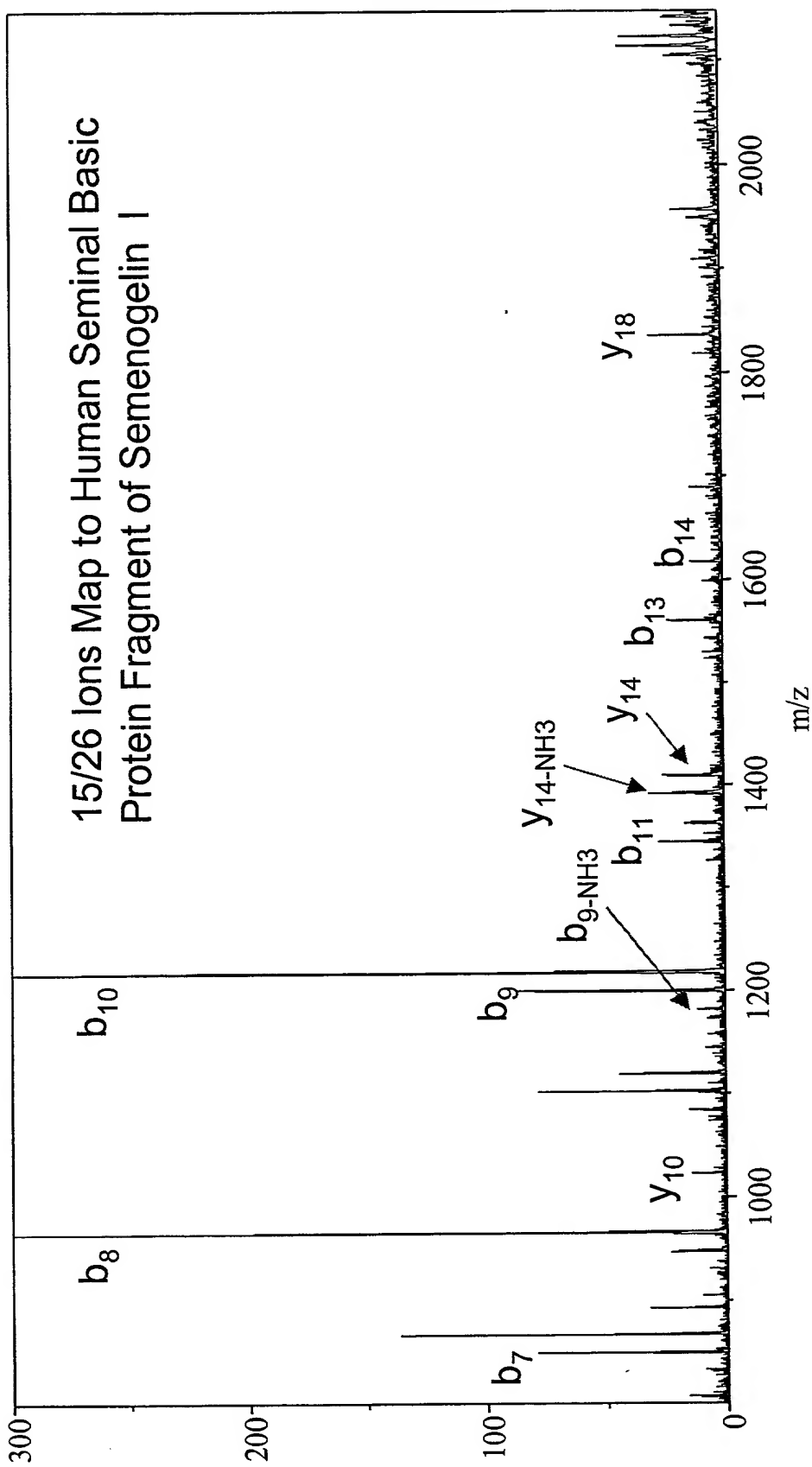


FIG. 7

Sheet 8 of 27

Retentate Mapping of a Peptide Map on a Reverse Phase ProteinChip® Array

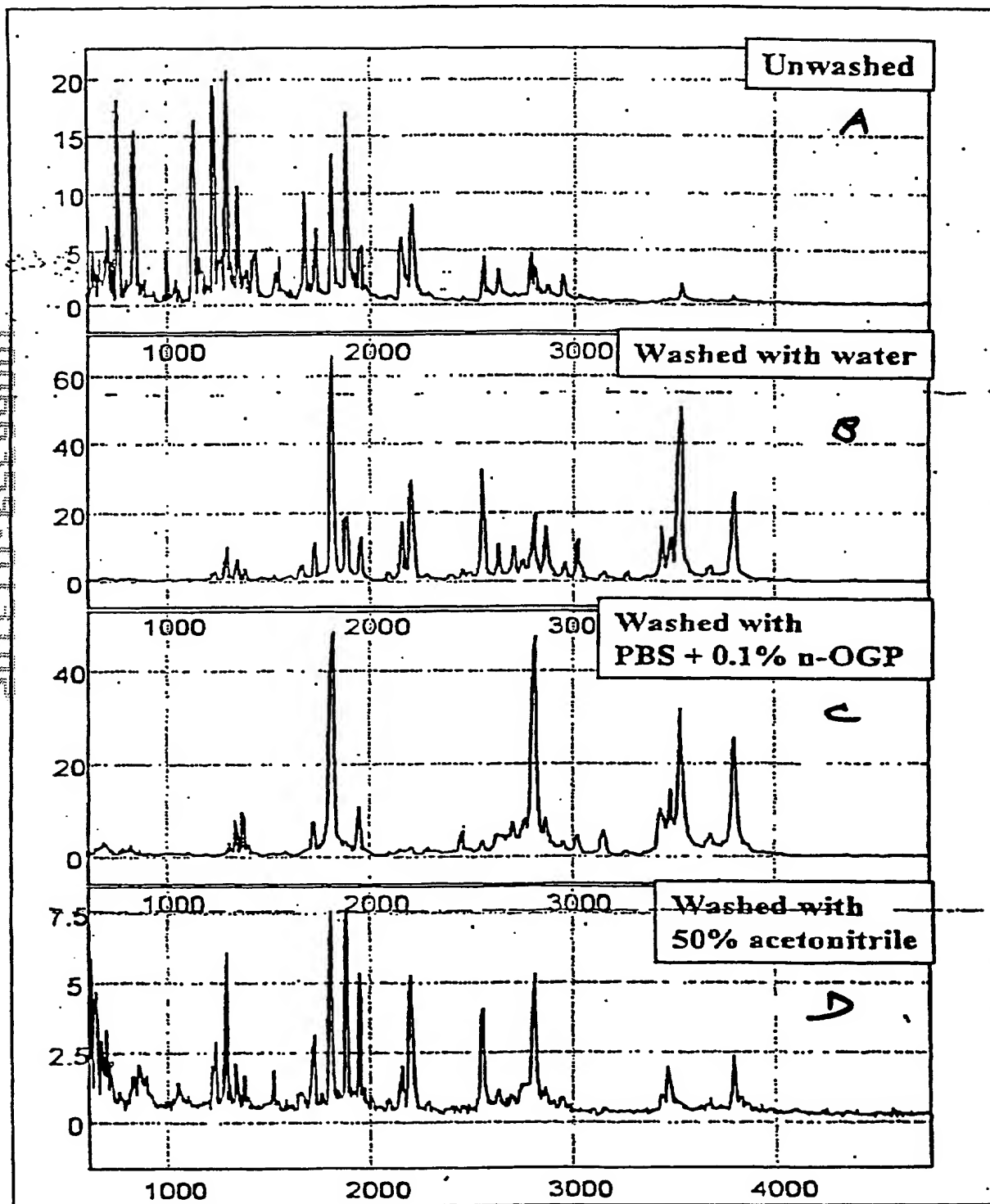


FIG. 8

Sheet 9 of 27

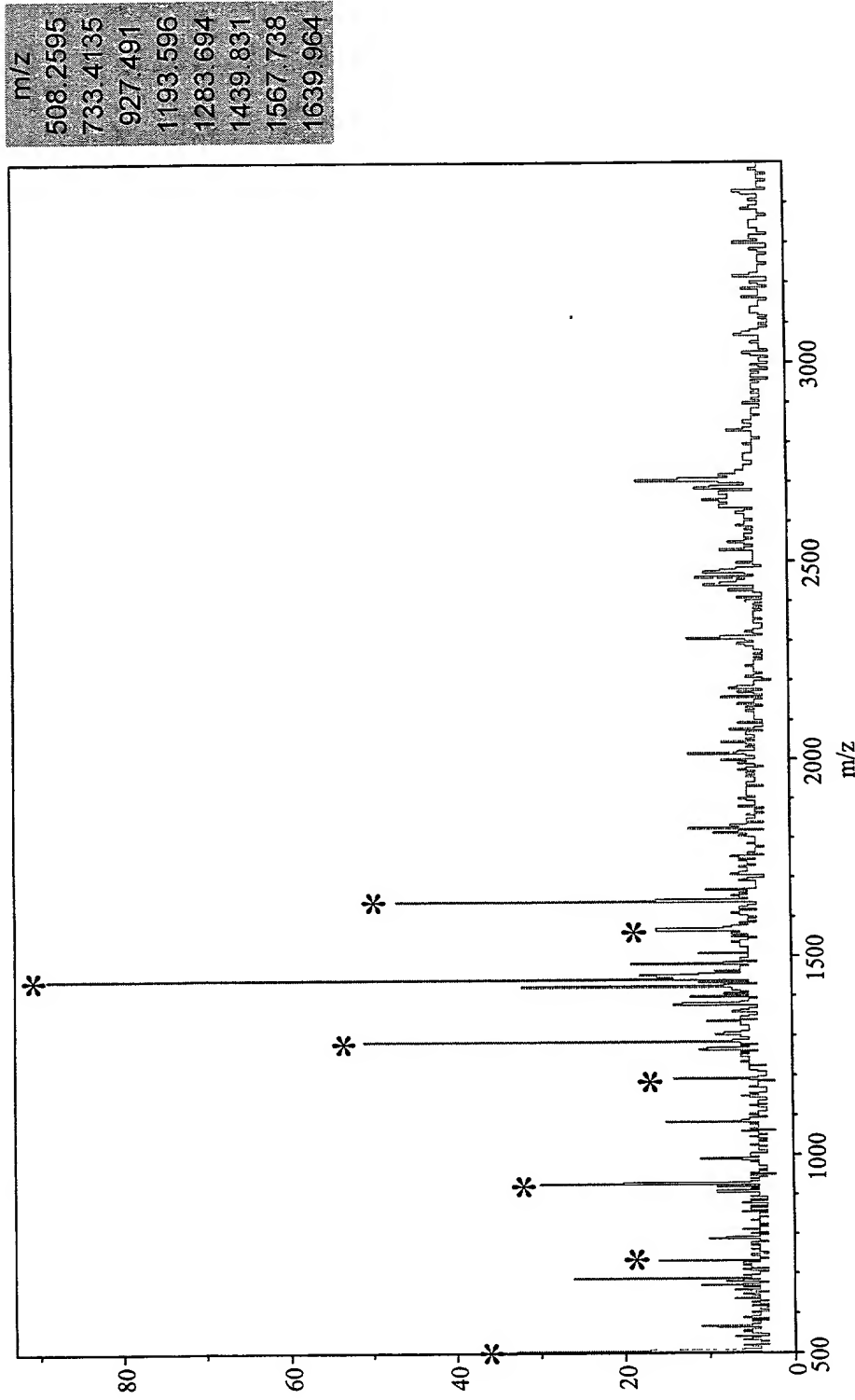


FIG. 9

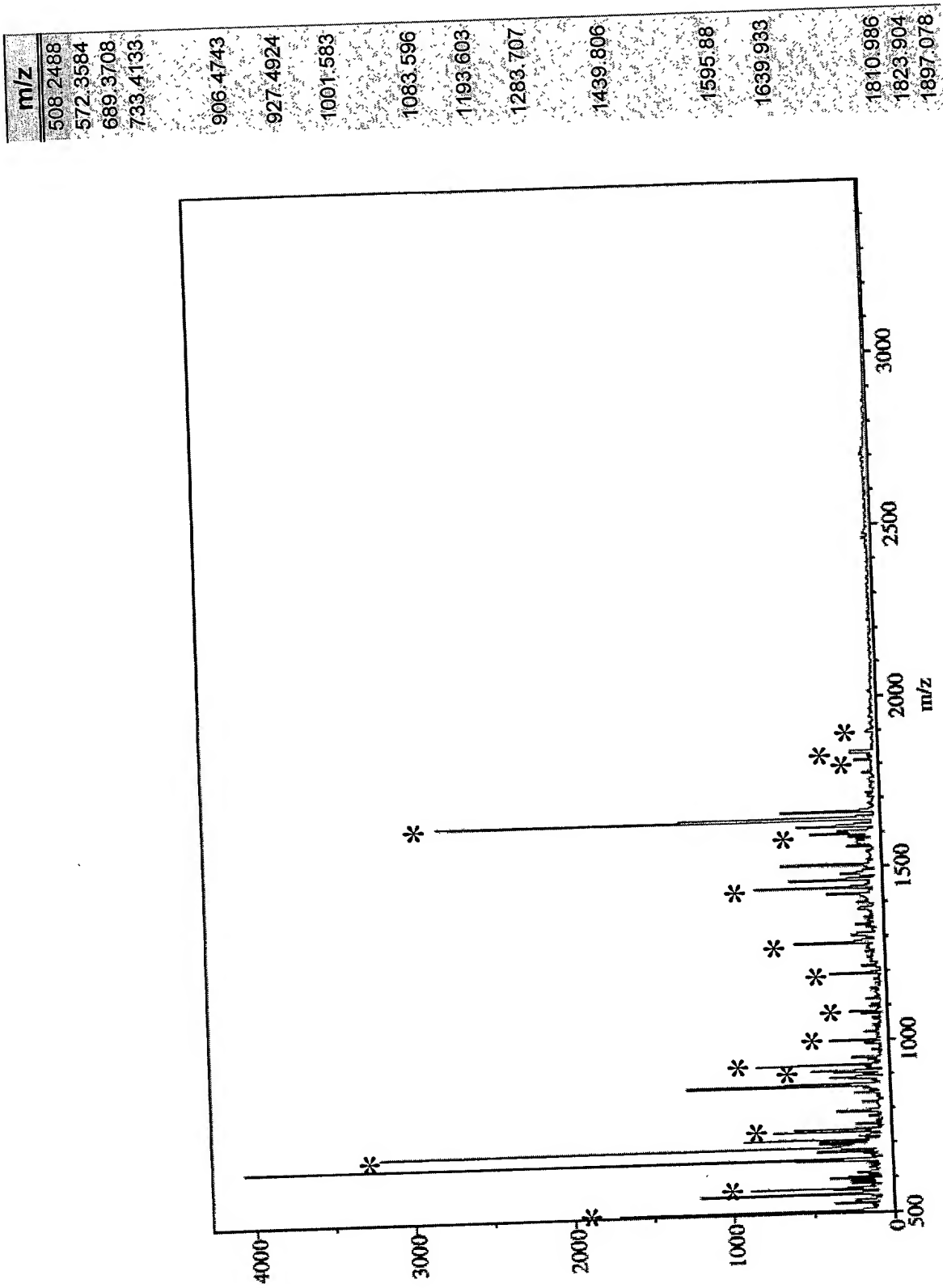


FIG. 10

Sheet 11 of 27

Fragment	esidues	Cal. M/Z	pI	H2O	pH3	pH4	pH5	pH6	pH7	pH8	pH9	All Conditions
30	204-207	K/F...R/A	6	508.2624	508.2624	508.2523	508.2507	508.2488	508.2477	508.247	508.2471	508.2518
27-28	194-197	R/Q...R/C	12	572.3638	572.3638	572.3628	572.3623	572.3584	572.3585	572.3576	572.3581	572.3628
32	211-216	K/A...R/L	9.8	689.3766	689.376	689.3753	689.3753	689.3708	689.3693	689.3692	689.37	689.3728
26	187-193	K/V...R/Q	9.7	733.4199	733.4219	733.421	733.4202	733.4133	733.4159	733.4093		733.4208
66	457-463	R/L...K/T	6.7			847.5087			847.5046		847.4989	841.4598
33-34	217-223	R/L...K/A	10	847.5069								847.5038
24-25	180-186	K/L...K/V	6.1	906.4826	906.4808	906.4814	906.4786	906.4743	906.4594			906.4718
28-29	196-203	R/L...K/F	9.5			918.5239						918.5188
19	137-143	K/Y...R/R	6	927.4875	927.488	927.497	927.4961	927.4924	927.4889	927.4866	927.4905	927.4938
25-26	185-193	R/E...R/Q	8.8	990.5469		990.5759						990.5578
31-32	208-216	R/A...R/L	11	1001.61	1001.592	1001.592	1001.585	1001.583	1001.568	1001.581	1001.582	1001.5888
26-27	187-195	K/V...R/L	12									
69	473-481	K/C...R/R	6									
19-20	137-144	K/Y...R/H	8.6	1083.597	1083.602	1083.602	1083.601	1083.596	1083.59	1083.605	1083.575	1083.5948
29-30	198-207	R/C...R/A	8.2	1138.579	1138.578							1138.5678
36893	36901	-D...R/F	6.9	1193.609		1193.611	1193.595	1193.603	1193.596	1193.593	1193.56	1193.6018
36954	37215	R/F...K/G	5.4	1249.624								1249.6208
50	336-346	R/H...R/L	6.7	1283.726	1283.714	1283.714	1283.712	1283.707	1283.701	1283.697	1283.712	1283.7108
55	377-387	K/H...K/Z	5.3	1305.707	1305.707	1305.689	1305.693					1305.7158
8	65-76	K/S...K/V	5.3									1362.6718
49-50	335-346	R/R...R/L	8.7	1439.812	1439.815	1439.819	1439.812	1439.806	1439.801	1439.794	1439.81	1439.8118
18-19	133-143	K/F...R/R	8.5									
68-70	470-482	K/V...R/P	10.8									
48	322-334	K/D...R/R	4.4	1567.756								1567.7428
50-51	336-349	R/H...K/E	8.6	1595.906	1595.908	1595.882	1595.891	1595.88			1595.895	1595.9268
74-77	519-533	K/Q...K/P	10								1777.12	1777.1058
59-60	411-425	R/K...R/S	8.7	1639.943	1639.942	1639.944	1639.94	1639.933	1639.928	1639.928		1639.9378
65	443-456	R/M...R/L	4.4									
35-36	224-238	K/A...K/V	4.7	1692.942	1692.942							1692.9418
33-35	217-231	R/L...K/L	8.5	1750.952	1750.951	1750.961						1750.9738
66-68	457-472	R/L...K/C	8.2	1811.035	1811.035	1811.015	1811.035	1810.986	1811.007	1810.992		1811.0088
70-71	482-497	R/R...K/A	6	1823.912	1823.912	1823.907	1823.9	1823.904				1823.8998
60-61	412-429	K/V...K/V	8.7					1897.078				1897.0748
14-16	115-131	K/L...K/K	4.4	1962.931	1962.931							1962.9478
47-48	316-334	K/N...R/R	4.7	2301.035	2301.035							2301.0818
36895	36911	-D...K/G	6.3			2441.13						
37-39	239-260	K/V...K/Y	4.9									2441.0998
47-49	316-335	K/N...R/H	6.1									2457.1828
14-18	115-136	K/L...K/Y	6.2									
63-65	434-456	R/C...R/L	4.9									2701.2448
36-39	232-260	K/L...K/Y	4.9									3211.5538
51-54	347-376	R/L...K/H	4.7									
Total Peptides				14	24	23	16	16	14	12	12	34
Sequence Coverage (%)				18	34	29	21	20	17	15	9	45

FIG. 11

Sheet 12 of 27

fragment residues	Cal. M/Z	pI	H2O	pH3	pH4	pH5	pH6	pH7	pH8	pH9	All Conditions
30	204-207	K/F...R/A	508.2518	508.2478	508.2457	508.2456	508.2476	508.2478	508.246	508.2455	508.25183
27-28	194-197	R/Q...R/C	572.3628	572.3539	572.352	572.3545	572.3527				572.36283
32	211-216	K/A...R/L	689.3728	689.3697	689.3688	689.3688	689.3702	689.3701	689.3629	689.3663	689.37283
26	187-193	K/V...R/Q	733.4208	733.4115	733.4089	733.4003					733.42083
66	457-463	R/L...K/T	841.4598								
33-34	217-223	R/L...K/A	847.5038								
24-25	180-186	K/L...K/V	906.4718	906.4688	906.4671	906.4667	906.4706	906.4697	906.464	906.4672	906.47183
28-29	196-203	R/L...K/F	918.5188	927.483	927.4887	927.4859	927.4861	927.4802	927.4855	927.4863	927.48383
19	137-143	K/Y...R/R	927.4938								
25-26	185-193	R/E...R/Q	990.5578	1001.578	1001.582	1001.566		1001.571			1001.58883
31-32	208-216	R/A...R/L	1001.589								
26-27	187-195	K/V...R/L	1017.58								
69	473-481	K/C...R/R	1024.455	1024.445		1024.446					1024.45483
19-20	137-144	K/Y...R/H	1083.595	1083.589	1083.591	1083.588	1083.584	1083.593		1083.581	1083.59483
29-30	198-207	R/C...R/A	1138.568								
36893	36901	-D...R/F	1193.602	1193.599	1193.596	1193.594	1193.599	1193.599	1193.596	1193.596	1193.60183
36954	37215	R/F...K/G	1249.621	1249.62				1249.592	1249.605	1249.607	1249.62083
50	336-346	R/H...R/L	1283.711	1283.706	1283.678	1283.694	1283.687	1283.702	1283.693	1283.695	1283.71083
55	377-387	K/H...K/Z	1305.716	1305.704							1305.71583
8	65-76	K/S...K/V	1362.672								
49-50	335-346	R/R...R/L	1439.812	1439.808	1439.795	1439.795	1439.797	1439.799	1439.792	1439.792	1439.81183
18-19	133-143	K/F...R/R	1445.758								
68-70	470-482	K/V...R/P	1508.767								
48	322-334	K/D...R/R	1587.743	1587.743							
50-51	336-349	R/H...K/E	1595.927	1595.906	1595.906	1595.889	1595.885	1595.892	1595.892	1595.892	1587.74283
74-77	519-533	K/Q...K/P	1777.106								1595.92683
59-60	411-425	R/K...R/S	1639.938	1639.935	1639.923	1639.923	1639.933	1639.934	1639.942	1639.927	1777.10583
65	443-456	R/M...R/L	1667.813								1639.93783
35-36	224-238	K/A...K/V	1692.942	1692.942							1692.94183
33-35	217-231	R/L...K/L	1750.974								
66-68	457-472	R/L...K/C	1811.009	1811.03	1810.998	1810.998	1810.989	1810.991			1811.00883
70-71	482-497	R/R...K/A	1823.9								1823.89983
60-61	412-429	K/V...K/V	1897.075								
14-16	115-131	K/L...K/K	1962.948	1962.954							1962.94783
47-48	316-334	K/N...R/R	2301.082	2301.074							2301.08183
36895	36911	-D...K/G	2424.205	2424.192							2424.20483
37-39	239-260	K/V...K/Y	2441.1								2441.09983
47-49	316-335	K/N...R/H	2457.183								
14-18	115-136	K/L...K/Y	2609.306								
63-65	434-456	R/C...R/L	2701.245								
36-39	232-260	K/L...K/Y	3211.554	3211.56				3211.513			3211.55383
51-54	347-376	R/L...K/H	3420.579								
Total Peptides											26
Sequence Coverage (%)											37

FIG. 12

Sheet 13 of 27

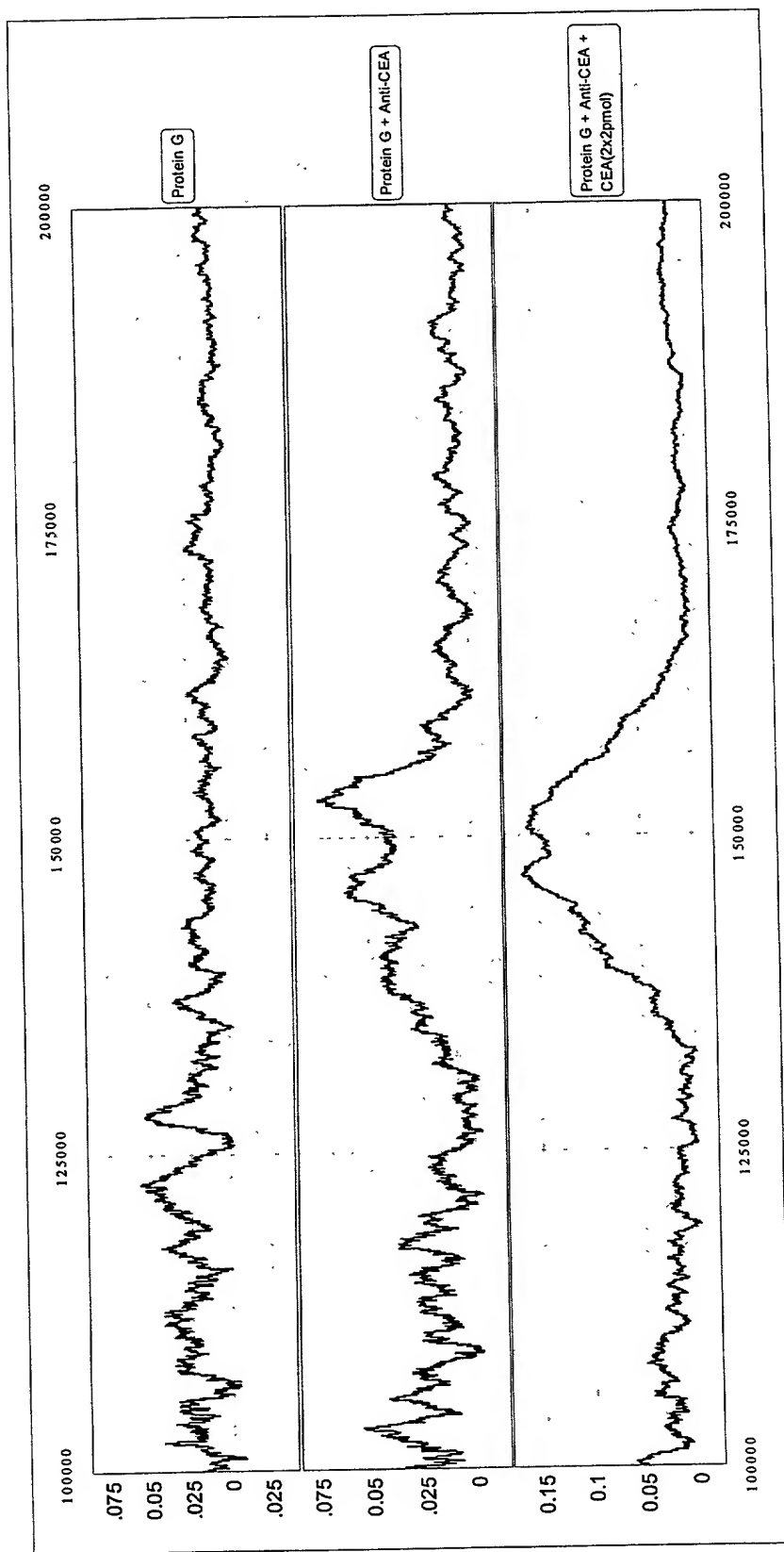


FIG. 13

Sheet 14 of 27

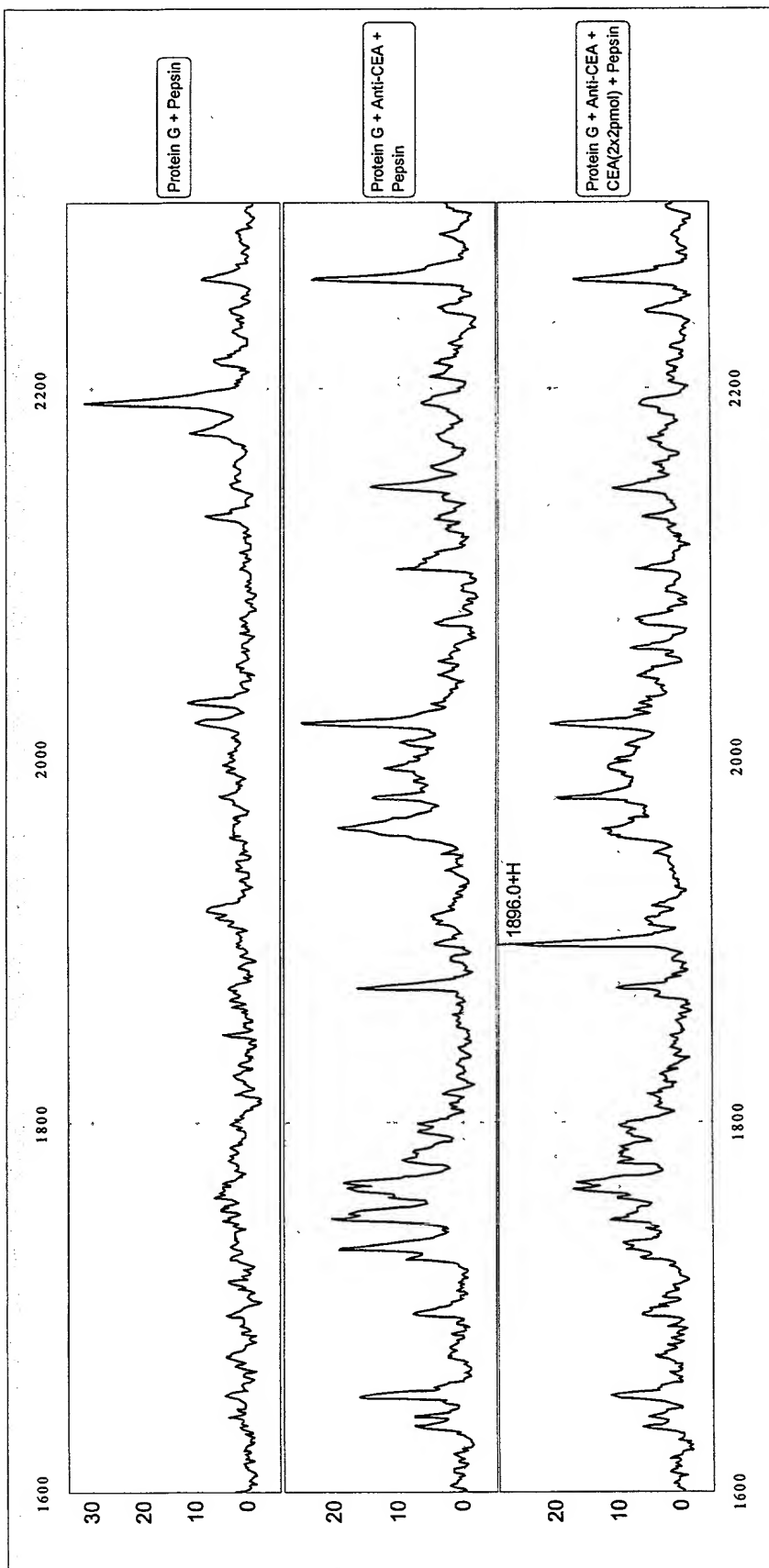


FIG. 14

Sheet 15 of 27

YVIGTQQATAYSGREPGP

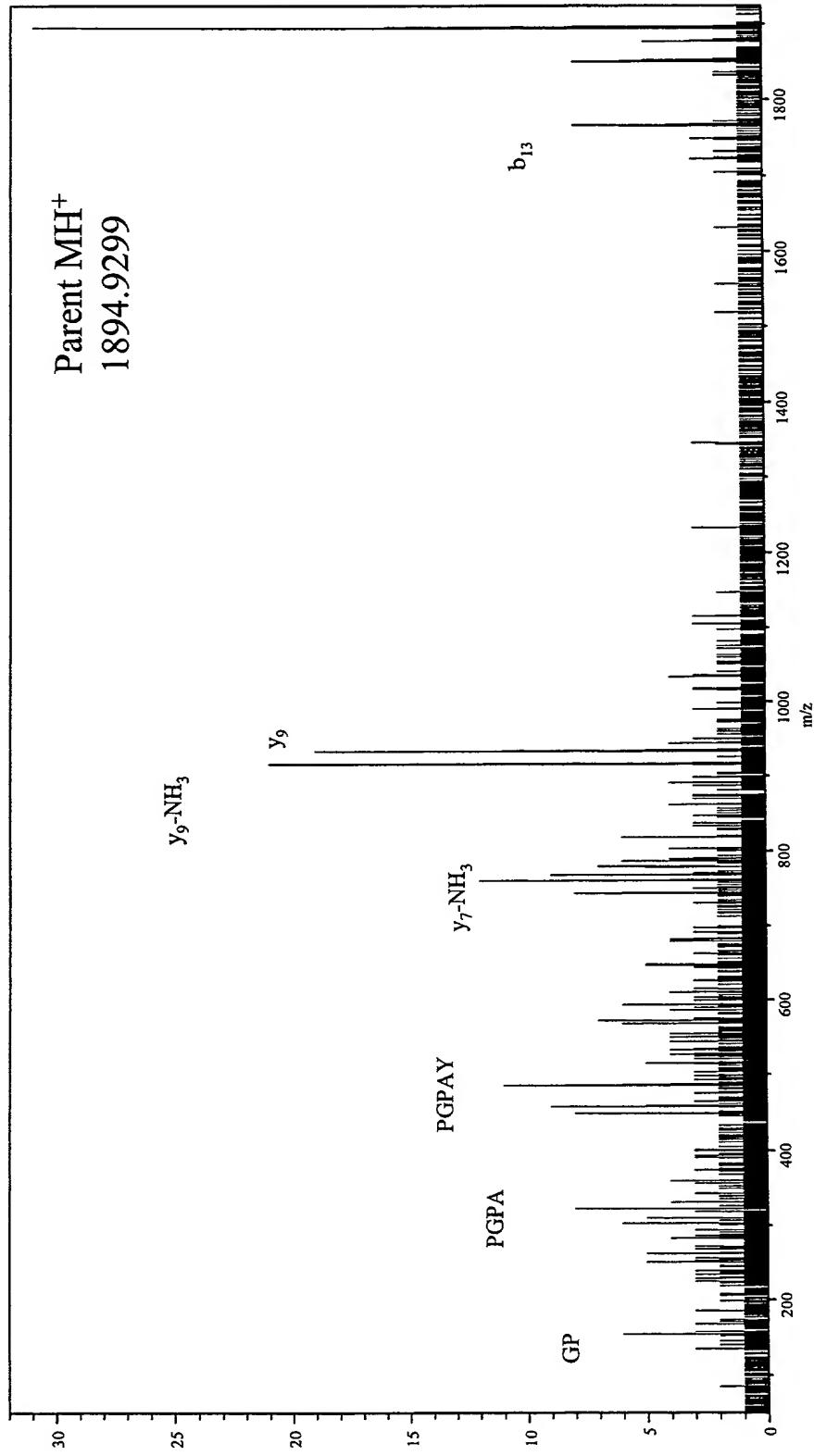


FIG. 15

Sheet 16 of 27

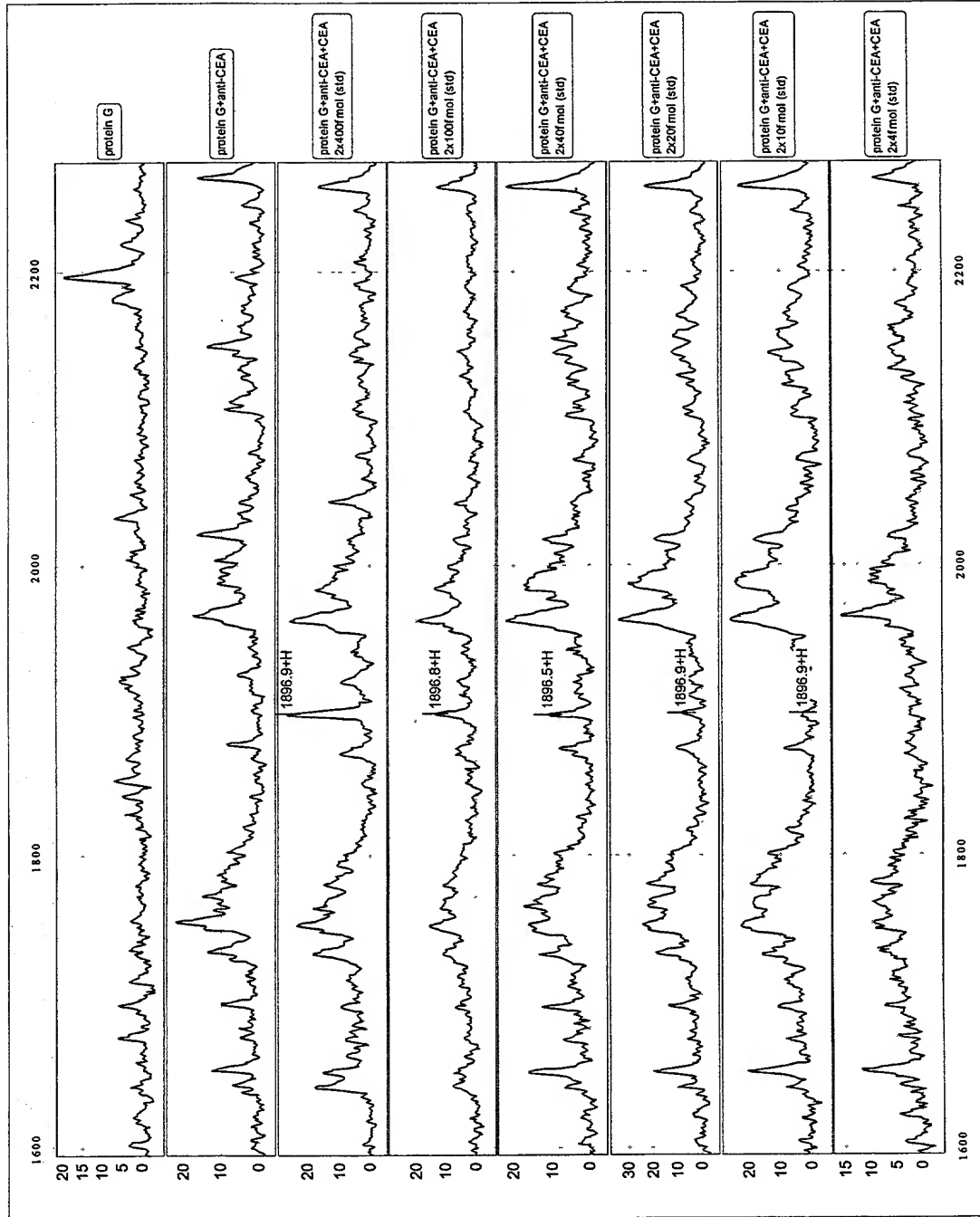


FIG. 16

Quantitative Antigen Capture

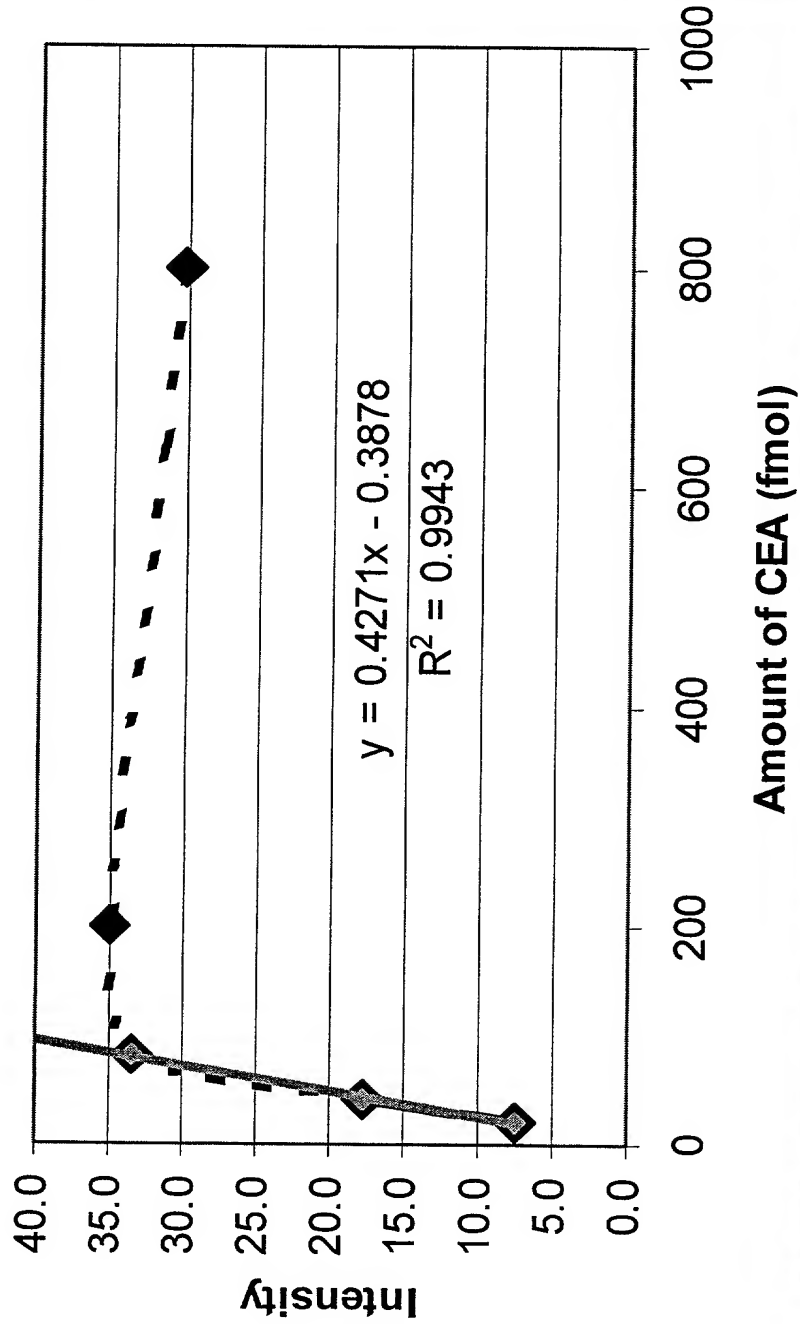


FIG. 17

Sheet 18 of 27

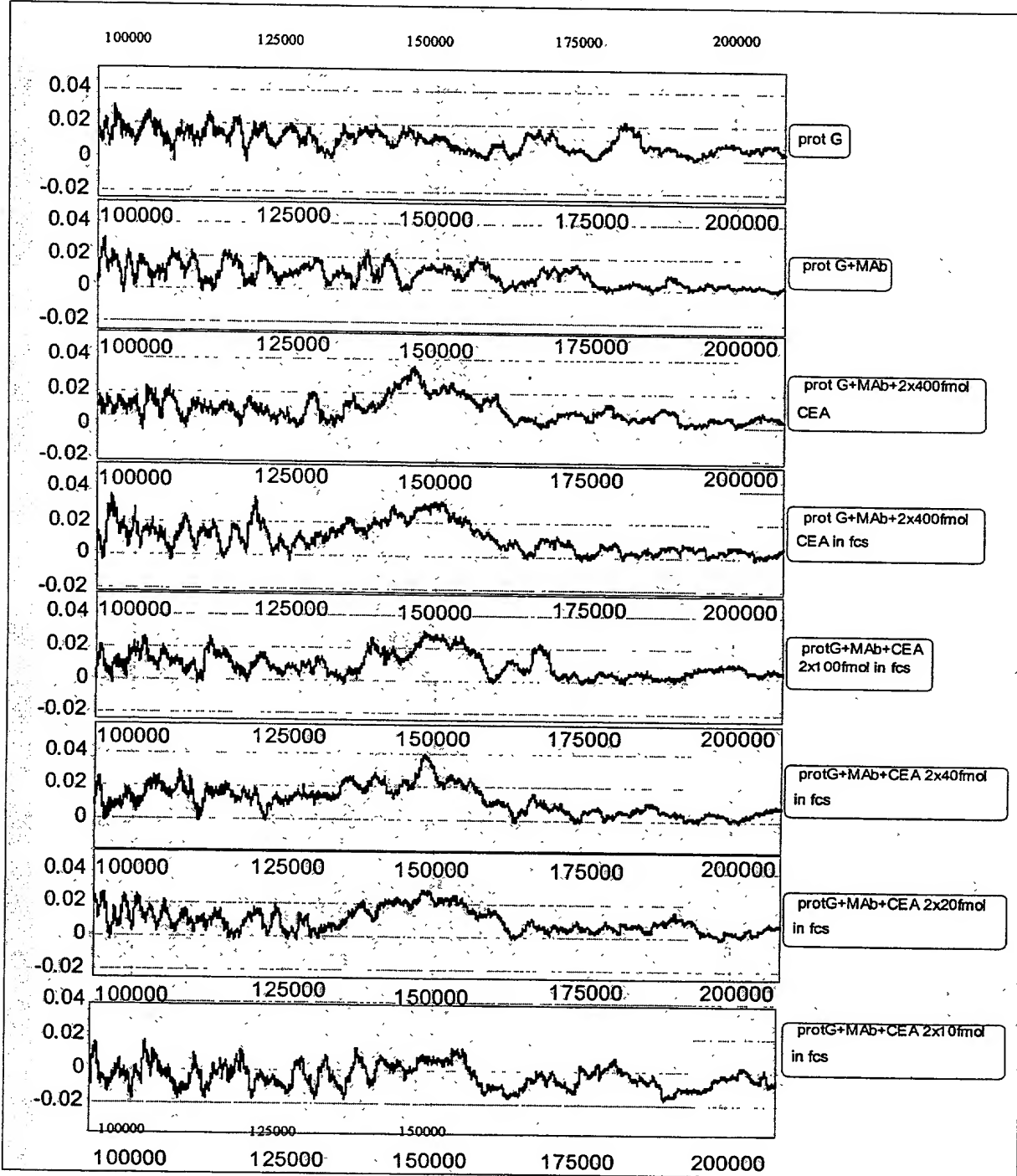


FIG. 18

Sheet 19 of 27

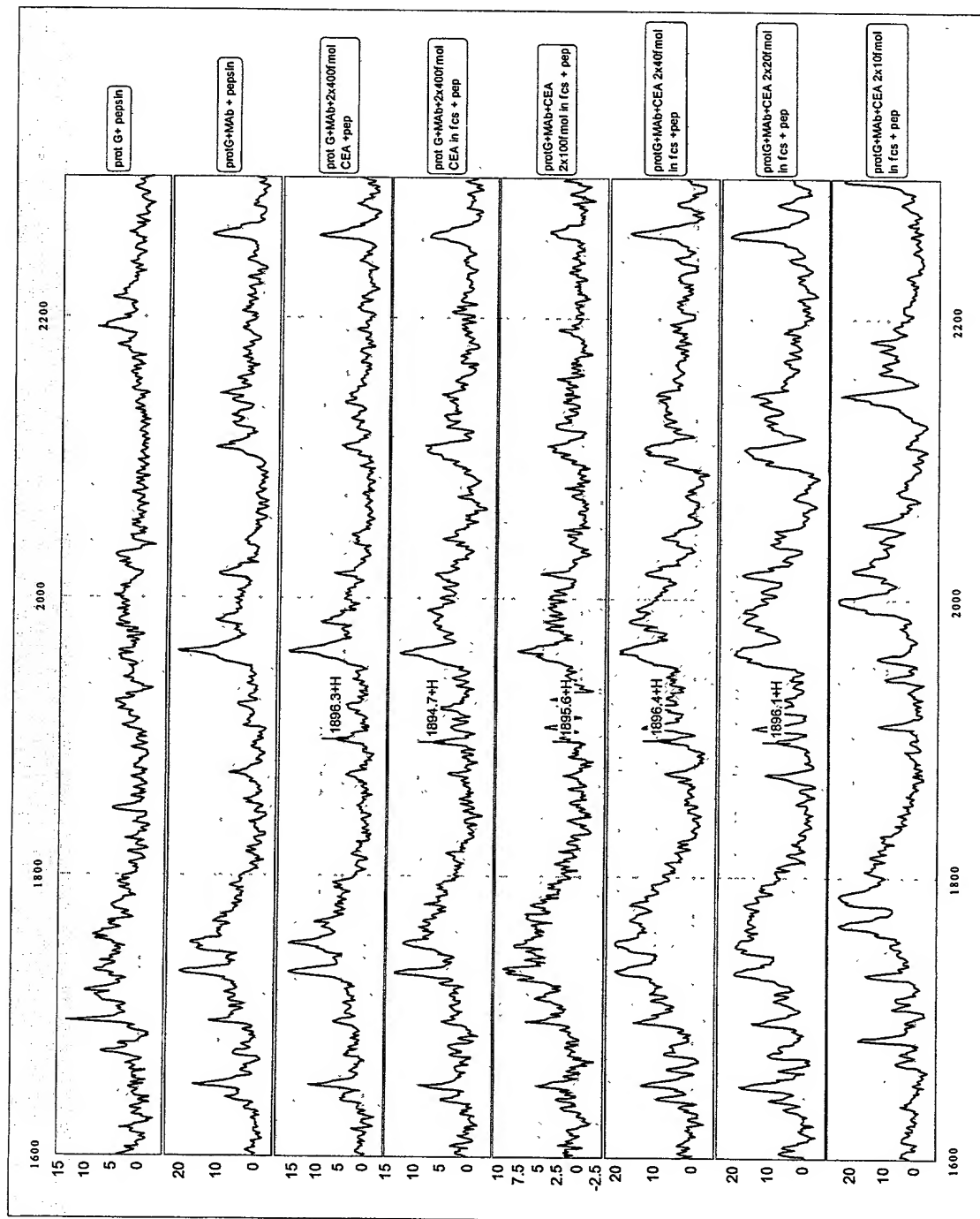


FIG. 19

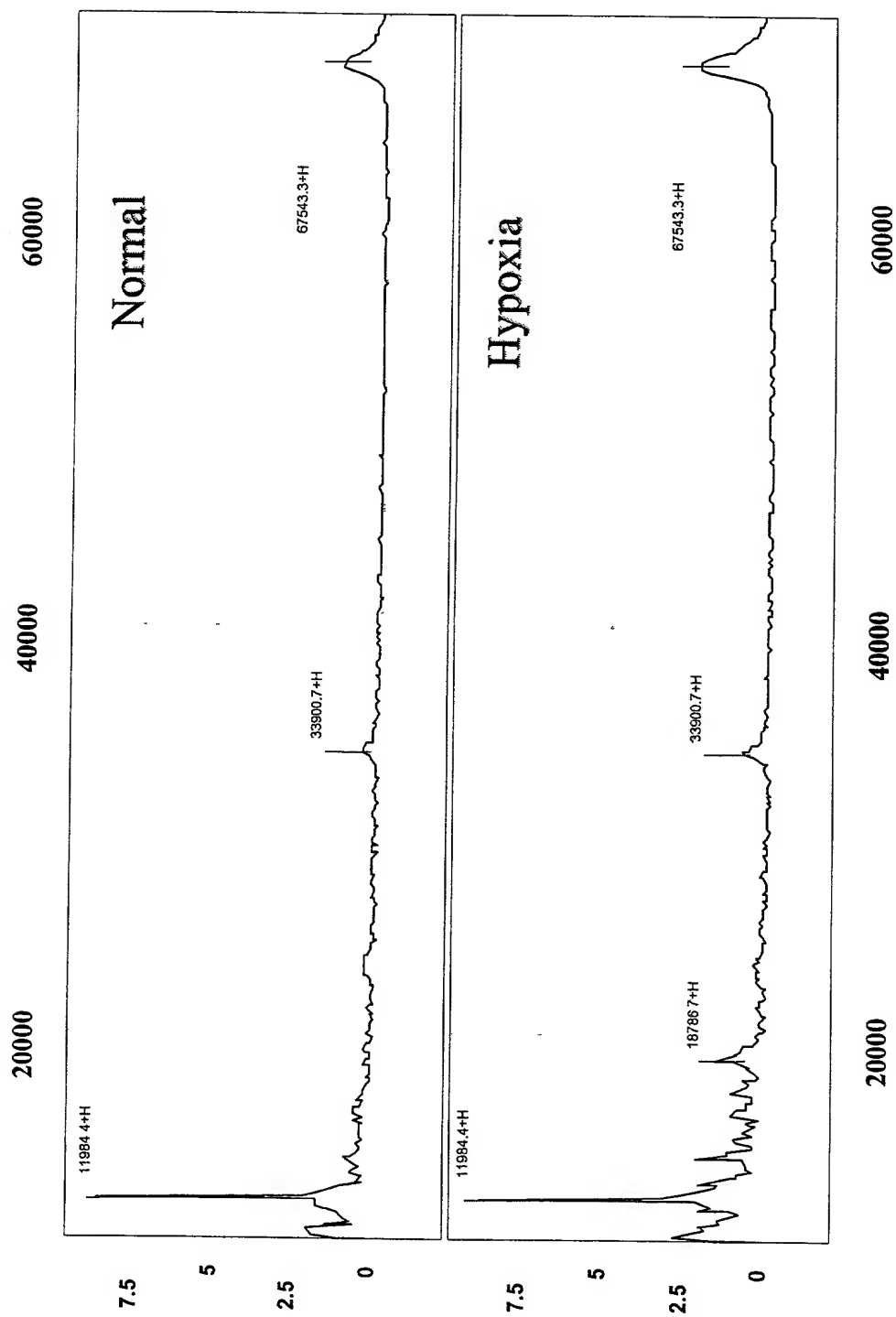
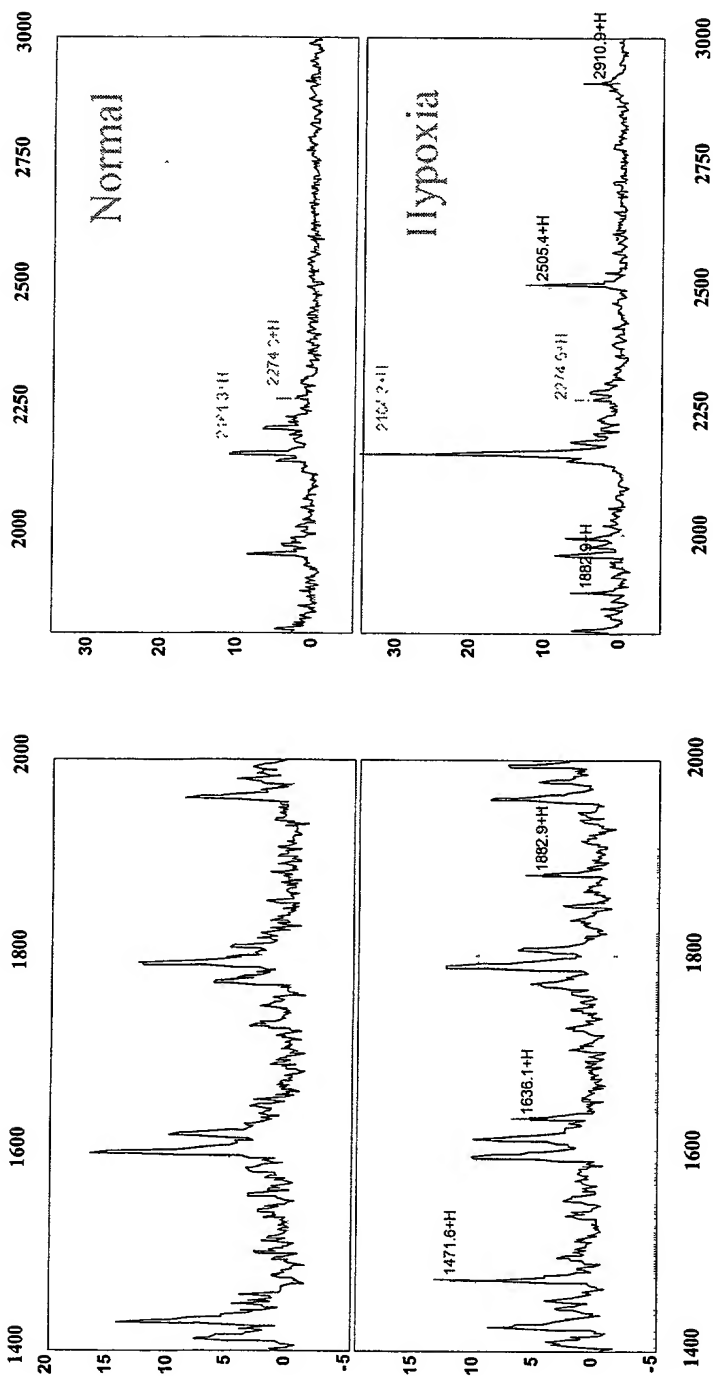


FIG. 20



Fragments generated from trypsin autolysis: 2164.3, 2274.6

FIG. 21

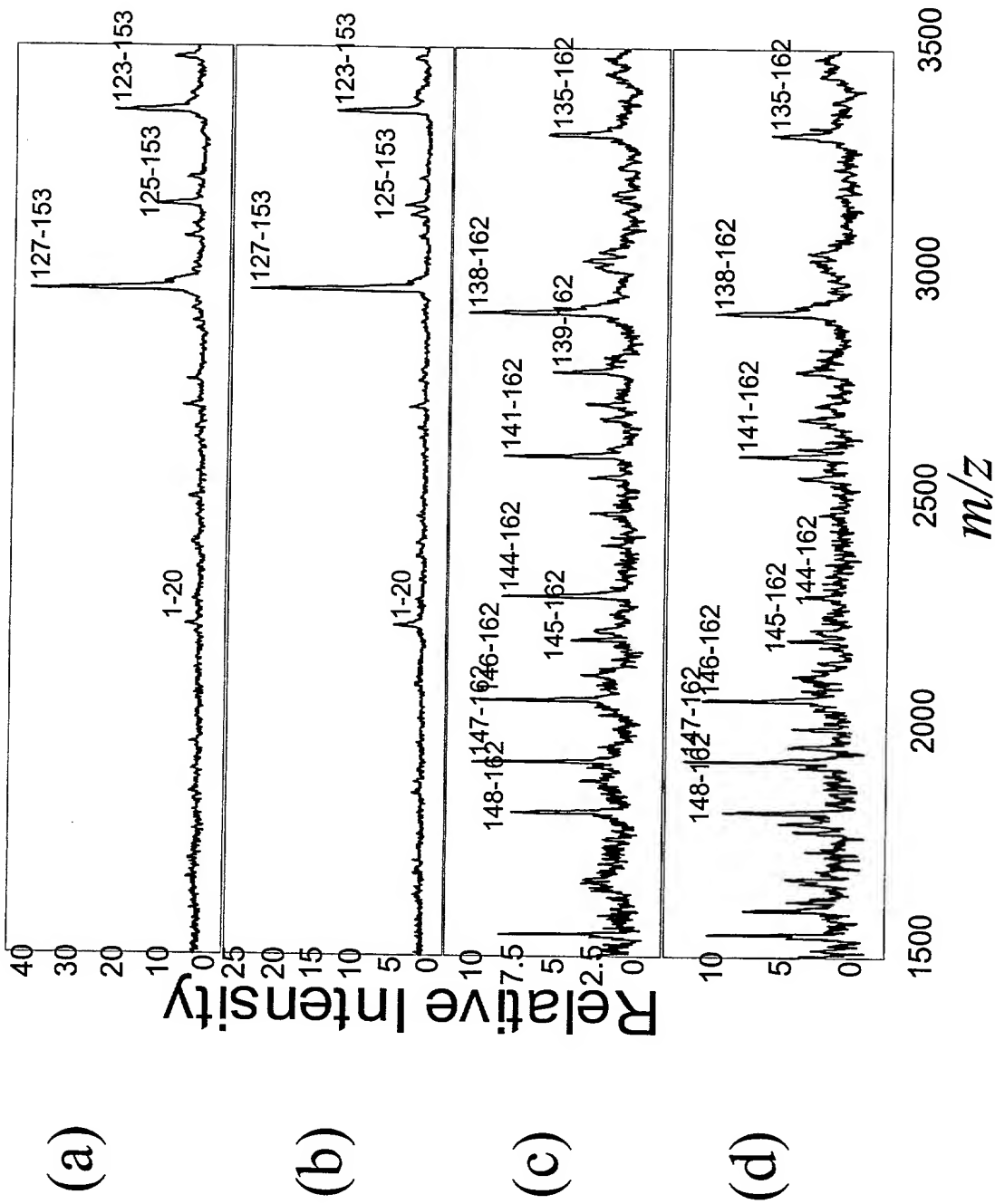


FIG. 22

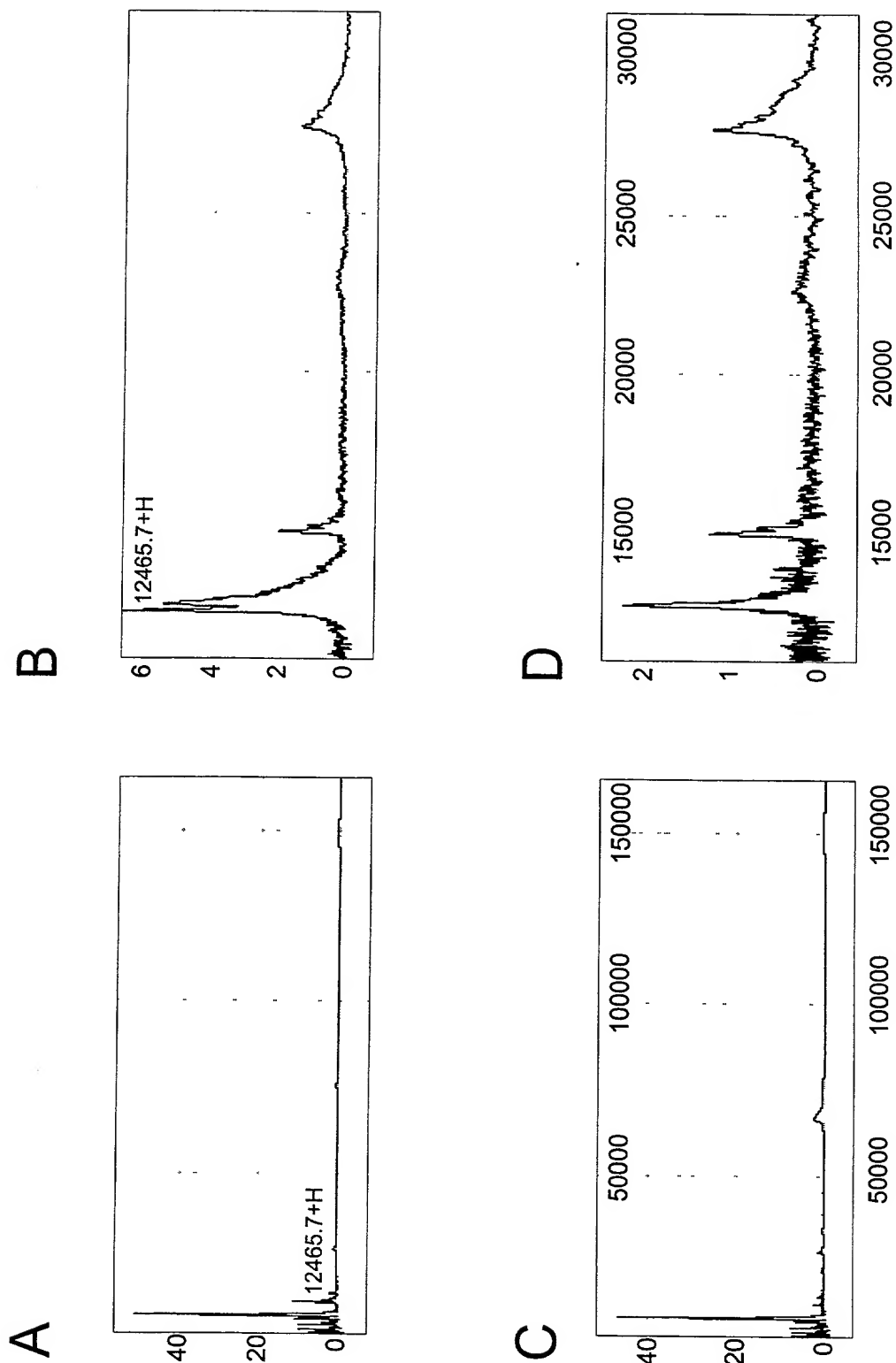


FIG. 23

Sheet 24 of 27

Single MS

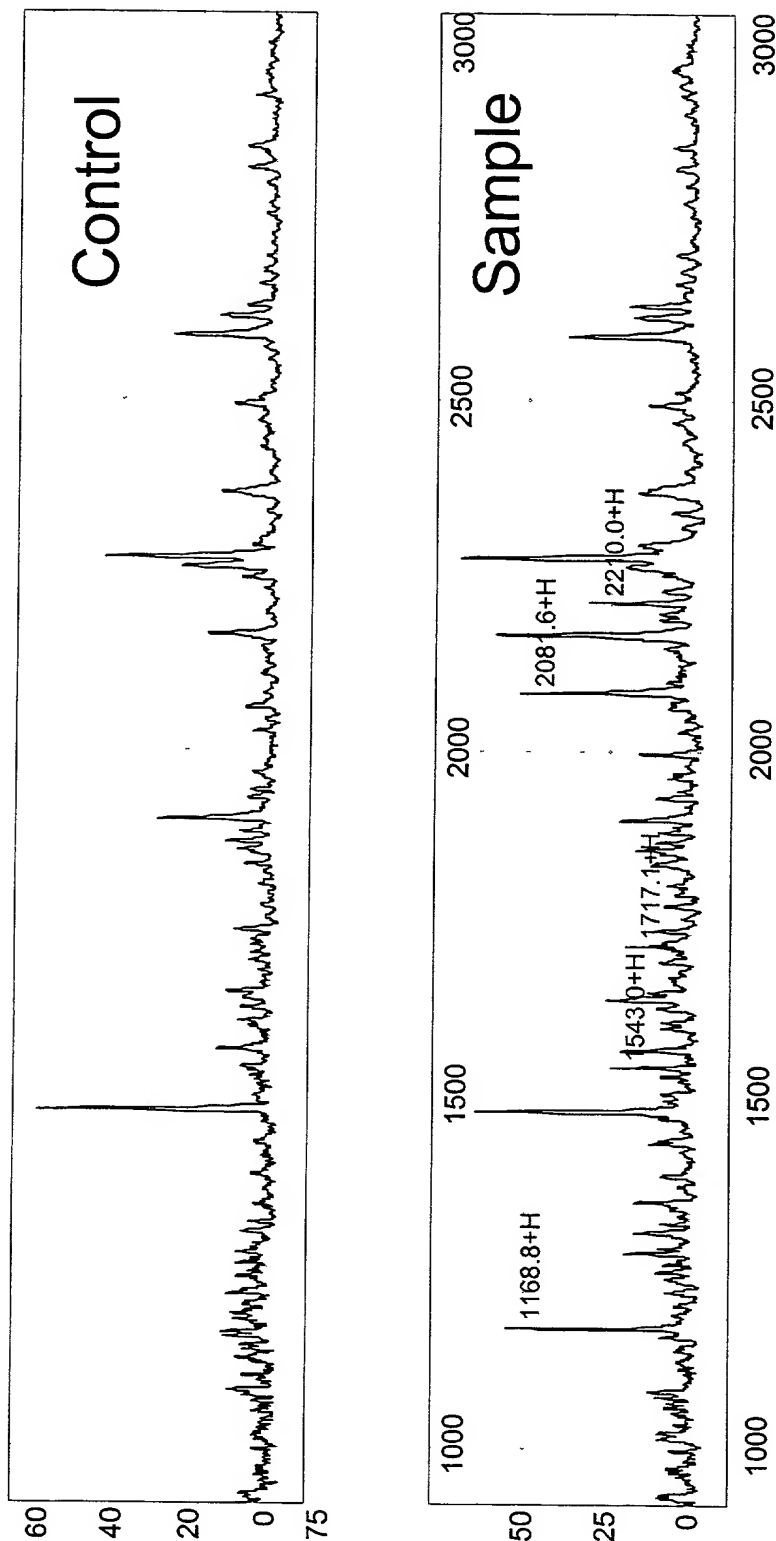
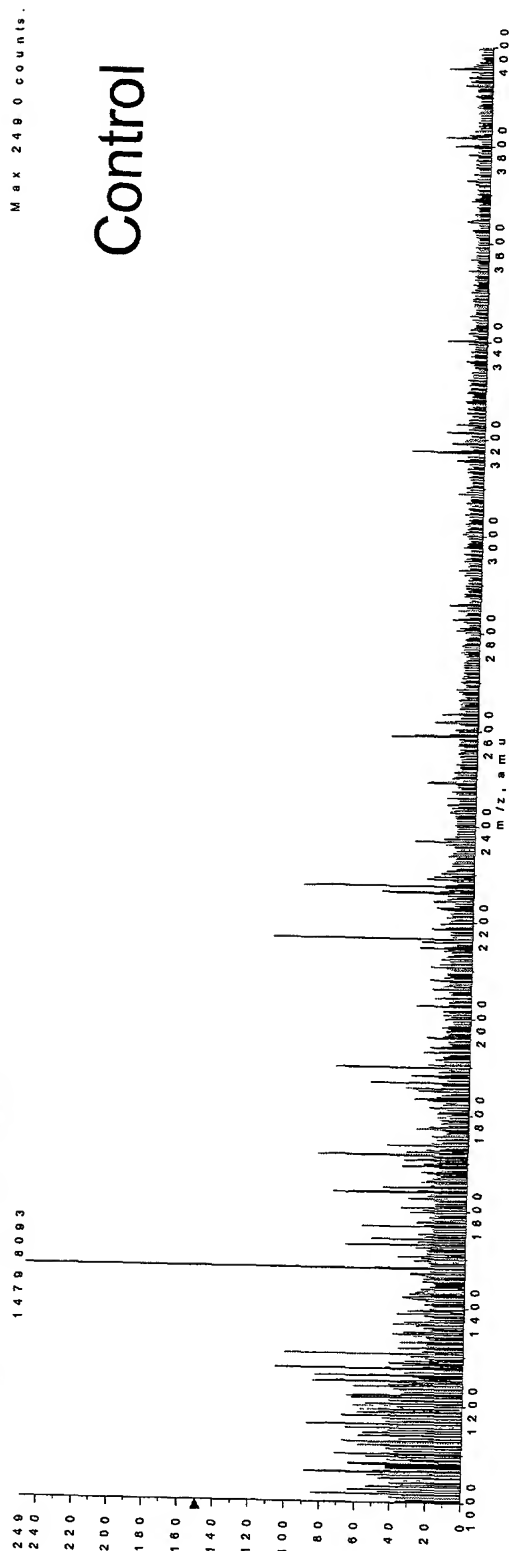


FIG. 24

QqTOF Tandem MS

A

TOF MS 60 MCA scans from FCS Trypsin Digest w/IF

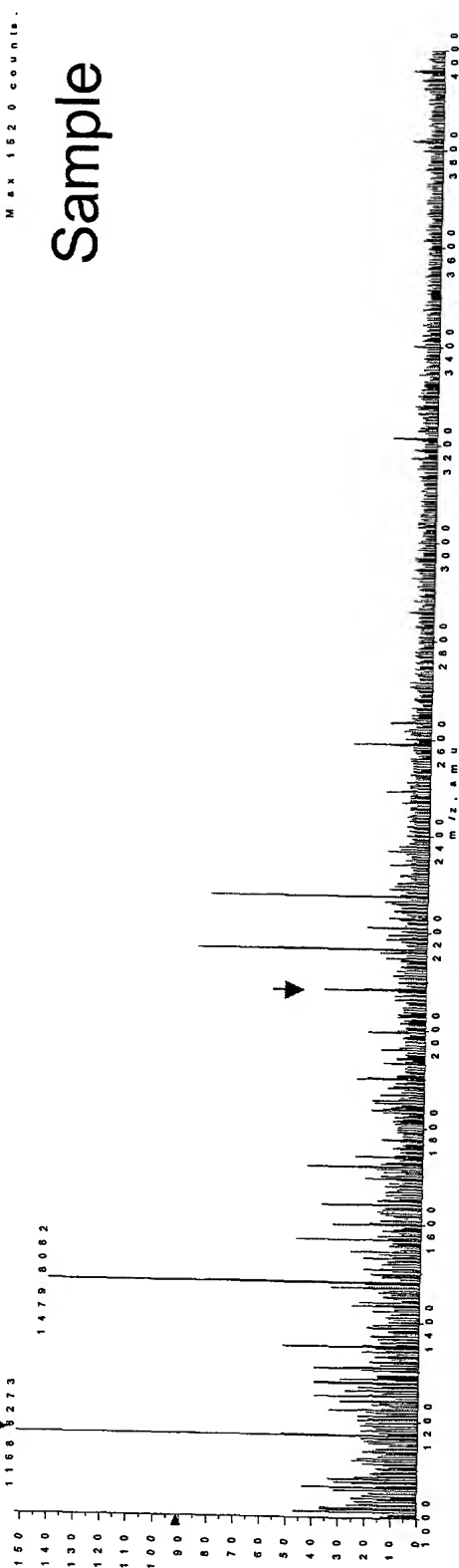


Control

Sheet 25 of 27

B

TOF MS 60 MCA scans from FCS-CytoC 6 spmolTrypsin Digest w/IF



Sample

FIG. 25

Sheet 26 of 27

1168 MS/MS

+TOF Product (1168.0): 97 MCA scans from FCS_CytoC 6.5pmol Trypsin Digest 1168MSMS.wiff

Max. 148.0 counts.

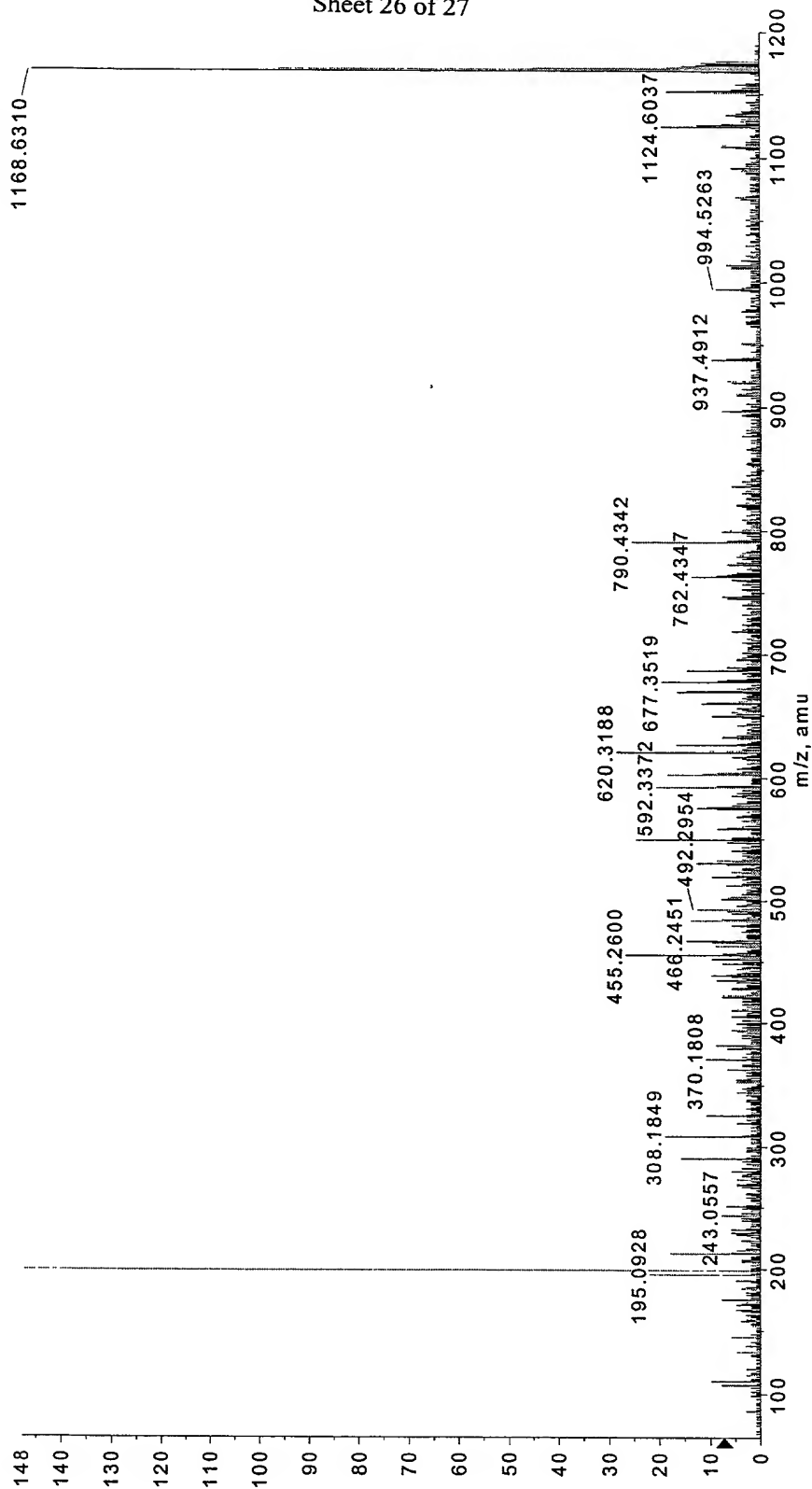


FIG. 26

MS-Tag Search Results

Press stop on your browser if you wish to abort this MS-Tag search prematurely
Sample ID (comment): Apo A-1 1040 AKPVLEDLR
Database searched: NCBIhm-12.5.2001
Full Molecular Weight range: 810480 entries
Full pI range: 810480 entries

Pro searches select 810480 entries

Ion Types Considered: a-NH3 b-H2O b-H2O y-NH3 y-H2O i-m

Search Peptide Masses Digest Max # Missed Cysteines Peptide
Mode are Used Cleavages Modified by N-terminus C-terminus
identity monoisotopic Trypsin 3 unmodified Hydrogen (H) Free-Acid (O H)

Number of sequences passing through parent mass filter: 18092

MS-Tag search selects 33 entries (results displayed for top 25 matches)

Parent mass: 1168.6310 (+/- 50.0000 ppm)

14 Fragment ions used in search: 175.10, 195.09, 243.06, 308.18, 455.26, 549.31, 620.32, 669.35, 677.35, 762.43, 790.43, 937.49, 994.53, 1108.60 (+/- 50.00 ppm)

Max # Unmatched Ions = 7

Result Summary

Rank	Unmatched Ions	Sequence	MT ⁺ Calculated Error (Da)	MT ⁺ Protein MTW (Da)/pI	Species	NCBIhm-12.5.2001 Accession #	Protein Name
1	3/14	(K)IGPNLHGLFGR(K)	1168.6227	7.1 11808.9 / 9.63	HOMO SAPIENS	15929398	(BC015130) cytochrome c
1	3/14	(K)IGPNLHGLFGR(K)	1168.6227	7.1 11888.0 / 9.52	UNREADABLE	14782885	>gi 4782885 ref XP_043240.1 (XM_043240) hypothetical protein XP_043240 [Homo sapiens]
1	3/14	(K)IGPNLHGLFGR(K)	1168.6227	7.1 11757.8 / 9.59	UNREADABLE	4139715	>gi 4139715 pdb 1G1W Solution Structure Of Reduced Horse Heart Cytochrome C, Nmr, Minimized Average Structure
1	3/14	(K)IGPNLHGLFGR(K)	1168.6227	7.1 11703.6 / 9.47	CHICKENS, HEART, PEPTIDE, 104 AA	914118	apocytochrome c
1	3/14	(K)IGPNLHGLFGR(K)	1168.6227	7.1 11825.8 / 9.54	HORSES, HEART, PEPTIDE, 104 AA	914117	apocytochrome c
1	3/14	(K)IGPNLHGLFGR(K)	1168.6227	7.1 11814.8 / 9.59	UNREADABLE	4139756	>gi 4139756 pdb 1WEJF Chain F, IgG1 Fab Fragment (Of B8 Antibody) Complexed With Horse Cytochrome C At 1.8 A Resolution
1	3/14	(K)IGPNLHGLFGR(K)	1168.6227	7.1 11474.3 / 9.61	GUINEA PIG, CERVANTINE SEQUENCES	483111	cytochrome c

FIG. 27